REMARKS

By the present communication, claims 31-32 and 49-51 are canceled, and claims 52-58 are added to define Applicants' invention with greater particularity. No new matter has been added and the new claims are fully supported by the specification and claims as originally filed. Claims 29-32 and 36-58 are pending. Support for the new claims includes but is not limited to:

Claim 52	Page 11, lines 6-9, 13-16.
Claim 53	Pages 5-6, 17-26.
Claim 54	Page 11, lines 9-12, 20-23.
Claim 55	Page 11, lines 6-9.
Claim 56	Page 11, lines 2-5, 25-30.
Claim 57	Pages 5-6, 17-26.
Claim 58	Page 5, line 9-10.

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

As a preliminary matter, Applicants thank the Examiner for courtesies extended to the Applicants' representative during a telephone interview on March 17, 2004. During the interview, the rejections of record and potential means for overcoming the rejections were discussed. No agreement was reached. However, Applicants' representative informed the Examiner that Applicants would file a Request for Continued Examination. The substance of Applicants' arguments is included in the remarks below.

I. Objections to Claims

The objections to claims 31, 36 and 46 as being dependent from rejected claims 29, 30 and 37 are respectfully traversed. Solely to advance prosecution, claim 31 has been canceled by the present communication, rendering the objection to claim 31 moot. As discussed

below (Section III), Applicants respectfully submit that claim 29, from which claims 36 and 46 depend, is patentable as written. Thus, claims 36 and 46 have been retained without amendment at present. Accordingly, applicants respectfully request the withdrawal of the objections to claims 31, 36 and 46.

II. Claim Rejections Under 35 U.S.C. § 112, first paragraph

Claims 29, 30, 32, 37-45, and 49-51 were rejected under 35 U.S.C. § 112, first paragraph as allegedly lacking adequate written description. The Office Action asserts:

> Applicants disclosure of the species of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, and 16 does not put applicants in possession of all possible lysine 2,3-aminomutases and thus applicants were not in possession of the claimed methods of use of all possible lysine 2,3-aminomutases.
>
> Newly amended claims 49-51 are rejected under U.S.C. 112, first paragraph because the recitation "containing purified lysine 2,3-aminomutase **other than that from Clostridium Subterminale SB4**" is not supported by the original specification at the time of filing. While applicants disclosure of a number of lysine 2,3-aminomutase from species other than Clostridium Subterminale SB4 is acknowledged applicants do not have support for the claimed genus of methods comprising the use of those lysine 2,3-aminomutase from species other than that from Clostridium Subterminale SB4.
>
> Office Action, p. 3-4 (emphasis in original).

During the telephone interview, the Examiner also stated "that the taught species were but one variable that went into consideration in deciding whether the claims were properly described." Examiner interview summary, mailed 4/7/2004. Claims 49-51 have been canceled and the rejection as it relates to those claims is moot. Applicants traverse the rejection as it relates to claims 29, 30, 32, and 37-45.

Applicants respectfully submit that the Examiner has not presented any basis for the present rejection beyond arguments directed to the taught species; applicants further submit that the specification and taught species fully support claims 29, 30, 32, and 37-45. First it is noted that the Examiner has allowed or would allow claims analogous to independent claims 29 and 30 (i.e. claims 47, 48, 31, 36, and 46) that contain all the same elements of the rejected claims but are limited to a single species of lysine 2,3-aminomutase. Thus, the Examiner has expressly acknowledged adequate written description for all the elements of independent claims 29 and 30 except the genus of lysine 2,3-aminomutases employed in the claimed methods. In other words, notwithstanding certain statements to the contrary, the present written description rejection relies on the argument "that applicants disclosure of eight species of the claimed genus is not sufficient to put applicants in possession of all enzymes having lysine 2,3-aminomutase activity, regardless of the source or structure of enzyme." *Id.*, page 3.

This argument is an insufficient basis to sustain the present written description rejection. According to the written description guidelines provided by the Patent Office, genus analysis depends primarily on two factors: 1) whether the art indicates substantial variation among the species within the genus of the claimed subject matter; and 2) whether a representative number of species is implicitly or explicitly disclosed in view of factor 1. (A copy of the relevant page of the Synopsis of Application of Written Description Guidelines is provided herewith.) The Examiner has failed to show any substantial variation among the species of the genus and therefore has failed to show that the disclosed species are not representative of the genus.

As described in the previous response (mailed 10/15/2003, pages 8-9), but not acknowledged by the Examiner, applicants' disclosure of eight previously unknown amino acid sequences for lysine 2,3-aminomutase enzymes provides a structure to function/activity relationship between the species that demonstrates applicants' possession of all possible lysine 2,3-aminomutases and therefore the possession of the claimed methods of use of all possible lysine 2,3-aminomutases. The disclosed amino acid sequences of eight species of lysine 2,3-

aminomutases readily reveal unique amino acid motifs that serve to identify all lysine 2,3-aminomutases to those skilled in the art. At least two unique motifs stand out: CxxxCRxCxR and (S, T)GG(D, E). These conserved sequences are present in each of applicants' disclosed sequences and allow those skilled in the art to easily identify as a lysine 2,3-aminomutase any protein containing them or any DNA sequence encoding them. Furthermore, these motifs allow one skilled in the art to clone and express any lysine 2,3-aminomutase of the invention as taught therein. See, e.g., Application, page 17, lines 9-22; page 26, lines 18-21.

Variation in the amino acid sequences of lysine 2,3-aminomutases is therefore irrelevant in light of the structure function relationship that exists in the taught species and that defines the entire genus of enzymes. The fact that applicants were able to identify the E. Coli lysine 2,3-aminomutase gene despite only 31% sequence identity with the gene from *Clostridium* convincingly supports this claim. *Id.* Thus, one of ordinary skill in the art may readily envision the recited genus of lysine 2,3-aminomutases in the claimed methods based on the structure-activity relationship provided above. Hence, the eight disclosed species are more than enough to represent the genus of lysine 2,3-aminomutases.

Moreover, new claims 53 and 57 recite more focused genuses of lysine 2,3-aminomutases that are also well supported by the disclosed species. For example, new claims 53 and 57 recite the use of prokaryotic lysine 2,3-aminomutases; each of the disclosed lysine 2,3-aminomutases are prokaryotic. Similarly, new claim 58 now expressly sets forth that the lysine 2,3-aminomutase used in the method of producing L-β-lysine contains an iron-sulfur cluster. As will be readily understood by those of skill in the art, iron-sulfur clusters are inherent to the lysine 2,3-aminomutases described in the specification and used in the claimed methods. (In fact one of skill in the art will readily recognize that the motif, CxxxCRxCxR, is a part of an iron-sulfur cluster.) Thus, claim 58 recites the use of only those lysine 2,3-aminomutases that contain iron-sulfur clusters. As noted above, Applicants have set forth seven species of lysine 2,3-aminomutases other than that from clostridium Subterminale SB4 and have provided extensive documentation regarding them, including the motifs discussed above. Accordingly, applicants

respectfully request withdrawal of the rejection of claims 29, 30, 32, and 37-45 under 35 U.S.C. §112, first paragraph, and submit that the new claims are fully supported by the specification.

III. Claim Rejections Under 35 U.S.C. § 102(b)

The rejections of claims 30, 38 and 39 under 35 U.S.C. § 102(b) as allegedly being anticipated by Chirpich I (J. Biol. Chem. 245, 1778-89, 1970) and, separately, by Chirpich II (Preparative Biochemistry 3, 47-52 (1973)) are respectfully traversed. Applicants' invention as defined by claim 30 distinguishes over both references by reciting a method of producing L-β-lysine, using a substantially pure lysine 2,3-aminomutase. As defined in the disclosure, "a substantially pure protein means that the desired purified protein is essentially free from contaminating cellular components, as evidenced by a single band following polyacrylamide-sodium dodecyl sulfate gel electrophoresis." Application, page 4, lines 19-22. Thus, a substantially pure lysine 2,3-aminomutase exhibits a single band following polyacrylamide-sodium dodecyl sulfate gel electrophoresis. As exemplified by the preparation of *Clostridial* lysine 2,3-aminomutase, the present invention provides substantially pure lysine 2,3-aminomutase. Ruzicka Affidavit, ¶ 5 and Appendix Figure (found in response mailed 10/15/2003). In contrast, Chirpich I and II simply do not teach methods of producing L-β-lysine using substantially pure lysine 2,3-aminomutase.

The enzyme activity isolated by Chirpich I was not substantially pure lysine 2,3-aminomutase as defined in applicants' disclosure. The Examiner asserts that "the lysine 2,3-aminomutase taught by Chirpich et al. I was 95% homogenous on the basis of disc gel electrophoresis and gel filtration." As one of ordinary skill in the art will readily understand, these nondenaturing gels do not have the resolution of the SDS-PAGE gels used by Applicants and cited in their definition of substantially pure in the specification. To prove this, Applicants submitted affidavits and exhibits showing the superiority of Applicants' preparation of substantially pure lysine 2,3-aminomutase. It is not a rebuttal of this evidence to simply ignore the differences in technology and cast aspersions on Applicants improvements to the techniques

of Chirpich. If the Examiner would find an explanation of the improvements made to the Chirpich techniques helpful to his analysis, Applicants can readily provide such in the form of an affidavit. Applicants respectfully request reconsideration of the above evidence.

The technique described by Chirpich II differs significantly from that claimed in the present application. Chirpich II describes the preparation of L-β-lysine "from L-lysine by the action of an enzyme, L-lysine-2,3-aminomutase, present in extracts of lysine-fermenting clostridia." Page 47. The extracts are prepared by disruption of wet packed cells by sonication in the presence of corundum powder. The cell debris is then centrifuged and used for the production of L- β -lysine without further purification. *Id.*, page 48. Those of skill in the art will understand that this minimally purified extract of lysine 2,3-aminomutase is not substantially pure and the Examiner has not offered any evidence to the contrary. Chirpich II cannot anticipate the present claims.

As noted previously, the present application presents additional non-chromatographic evidence of purity: activity. The present preparation of substantially pure lysine 2,3-aminomutase from *Clostridium*, when activated by the required cofactors in accordance with the methods of the invention, is ten times as active as the preparation of Chirpich I. Ruzicka Affidavit, ¶ 5. The far greater activity of substantially pure lysine 2,3-aminomutase in the claimed methods provides a real and distinct advantage in the efficient production of L-β-lysine over the methods disclosed by Chirpich I and II. Because the cited references fails to teach or suggest each and every element of the claimed methods, applicants respectfully request that the rejection of claims 30, 38 and 39 under 35 U.S.C. § 102(b) be withdrawn.

In addition, Applicants submit that new claims 54-56 recite additional elements not found in Chipich I and II. Neither of the cited references teaches the purification of the lysine 2,3-aminomutase in the presence of L-lysine (claim 53), in the presence of cobalt (claim 54), or under anaerobic conditions (claim 55) to give substantially pure lysine 2,3-aminomutase for use in the production of L-β-lysine. In fact, Chirpich I teaches away from the use of cobalt by stating that "Co^{2+} (0.1 mM) was strongly inhibitory (80%)" when testing various metal ions for their

effect on the activity of *Clostridial* lysine 2,3-aminomutase (page 1784). Hence, Applicants respectfully submit that claims 54-56 are also patentable over the cited art.

IV. Claim Rejections Under 35 U.S.C. § 103(a)

The rejection of claims 29, 37, 42, 43 and 45 under 35 U.S.C. § 103(a) as allegedly being obvious over Chirpich I (J. Biol. Chem. 245, 1778-89, 1970) and the rejection of claims 40 and 41 as allegedly obvious over Chirpich I in view of Rozzell (U.S. Patent No. 4,880,738) and Kusumoto (Tetrahedron Letters, 23, 2961-64) are respectfully traversed. As stated in Section 2143 of the MPEP,

> [t]o establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

It is respectfully submitted that the Examiner has not established a prima facie case of obviousness.

Applicants' invention as defined, for example, by claim 29 and its dependent claims distinguish over Chirpich I by reciting a method of producing L-β-lysine comprising (a) culturing a prokaryotic host cell comprising an expression vector that encodes lysine 2,3-aminomutase in the presence of L-lysine, wherein the cultured host cell expresses lysine 2,3-aminomutase, and (b) isolating L-β-lysine from the cultured host cells. Chirpich I fails to teach a single element of this claim. It fails to teach culturing a prokaryotic host cell. It fails to teach the use of an expression vector encoding lysine 2,3-aminomutase. It fails to teach that this is done in the presence of L-lysine. It fails to teach that the cultured host cell expresses lysine 2,3-aminomutase, and, finally, it fails to teach the isolation of L-β-lysine from the cultured host cell. Instead, Chirpich I discloses the purification of the naturally occurring lysine 2,3-aminomutase

from a crude cell extract. Thus, the cited reference bears little relation to the claimed method and the deficiency is not cured by the Examiner's statement that "[o]ne of ordinary skill in the art at the time of filing would have been motivated to use the purified lysine 2,3-aminomutase from *Clostridium* SB4 to generate antibodies against the enzymes such that the nucleic acid which encodes the enzymes could be isolated for further use in the recombinant production and characterization of lysine 2,3-aminomutase." Office Action, page 9. This statement is nothing more than an invitation to experiment.

Applicants respectfully submit that the Examiner has failed to show any suggestion or motivation in the cited art to make the claimed invention and has put forth an 'obvious to try' rationale in its place. As in the previous response, "what was 'obvious to try' was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it." MPEP Section 2145, X.B. (emphasis added). The issue is not whether it is obvious that recombinant techniques can be used to make useful proteins known to exist, the issue is whether it is obvious to produce L-β-lysine by the claimed methods.

Whether the claimed methods are obvious depends on more than whether lysine 2,3-aminomutase is a "useful protein." As shown above, the preparation of Chirpich was not a pure one. Thus, despite statements to the contrary in Chirpich, at the time of the invention one of ordinary skill in the art could not be sure that the lysine 2,3-aminomutase comprised a single protein. See Frey Declaration, ¶ 4 (see response mailed 10/15/2003). In fact, prior to applicants' invention, the literature relating to enzymes that use S-adenosylmethionine as a radical-initiating cofactor provided substantial grounds to believe that more than one protein was required for lysine 2,3-aminomutase activity because all other such enzymes were comprised of two proteins. See Frey Declaration, ¶ 5. This possibility actually taught away from making an attempt to clone lysine 2,3-aminomutase because of the uncertainty as to whether the experiment would yield an active enzyme. Until a gene has been heterologously cloned and expressed, the exact nature of the enzyme remains unknown. Thus, not only was there a substantial question of the possible

success of the method, there was little motivation to undertake it at the time. That nearly thirty years passed from the publication of Chirpich's article to the filing of the present application testifies to the lack of motivation to undertake the Examiner's suggested approach. Accordingly, a prima facie case of obviousness has not been established for claims 29, 37, 42, 43 and 45.

In the Final Office Action, the Examiner discounts the above arguments by stating that "applicants are reminded that this is an obviousness rejection, not a rejection based on anticipation," and "applicants argument is not found persuasive because as previously stated, Chirpich et al. teach the claimed method using naturally produced lysine 2,3-aminomutase" (page 10). Applicants first note that although the present rejection is an obviousness rejection, the Examiner is not relieved of his burden for showing the basis for each and every element of the claims in the prior art; as outlined above, applicants submit that the Examiner has failed to do this. In addition, the Examiner appears to ignore the evidence provided in the Frey affidavit regarding reasons that would make it unobvious for one of ordinary skill in the art to pursue the Examiner's suggested course of conduct. Also, the approximately 30 years that have passed between the publication of the Chirpich references and the present application was deemed as "not relevant." Applicants suggest that in combination the Frey affidavit and the lengthy period of time are highly persuasive and respectfully request that the Examiner reconsider both.

Moreover, new claim 52 recites the additional element of culturing the prokaryotic host cell in the presence of cobalt. No cited reference teaches this element, and Chirpich I actually teaches away from the inclusion of cobalt in the culture medium by stating: "Co^{2+} (0.1 mM) was strongly inhibitory (80%)" when testing various metal ions for their effect on the activity of *Clostridial* lysine 2,3-aminomutase (page 1784). Hence, Applicants respectfully submit that new claim 52 is patentable over the cited art.

The rejections of amended claim 40 and claim 41 are equally flawed. First, the cited art fails to teach the use of a substantially pure lysine 2,3-aminomutase. The higher activity of substantially pure lysine 2,3-aminomutase is a clear advantage in the production of L-β-lysine

by the claimed methods over the assay disclosed by Chirpich. Thus, the cited art does not teach or suggest each and every element of claims 40 and 41.

Furthermore, the cited art again fails to provide any impetus for one of ordinary skill in the art to do what the inventors have done. The Office Action fails to point to any teaching in Chirpich or Rozzell that suggests their combination; Kusomoto does not cure this deficiency. The previous Office Action (dated 7/15/2003) cites Kusumoto for its use of β-lysine in the synthesis of the antibiotic streptothricin F and states that "this synthesis method makes it possible to synthesize structural analogs of streptothricin which are necessary for the future studies of the relationship between structure and activity of the streptothricin antibiotic." Office Action, page 8. The Office Action then leaps to the conclusion that "[o]ne would have been further motivated to immobilize the recombinantly expressed lysine 2,3-aminomutase for use in a method of producing L-β-lysine for use in the synthesis of the antibiotic streptothricin and streptothricin analogs, so that the enzyme could be used repeatedly in a process of synthesizing L-β-lysine." Office Action, page 9. This statement is but a hindsight reconstruction of the invention.

Kusumoto does not teach or suggest the use of any enzyme for the production of β-lysine, or, indeed, the use of any enzyme for any purpose. Instead, Kusumoto reports a natural product synthesis that makes use of L-β-lysine. It cites a synthetic paper as its source for a synthetic derivative of L-β-lysine (footnote 9), but makes no other statement regarding this amino acid. Mere use of L-β-lysine in an antibiotic synthesis cannot provide motivation for the combination of Chirpich and Rozzell. The only suggestion for the claimed method comes from applicants' own disclosure. Hence, a prima facie case of obviousness has not been established for any claim. Applicants respectfully request that the rejections under 35 U.S.C. § 103(a) be withdrawn.

In the Final Office Action, the Examiner found the above arguments regarding claims 40 and 41 not persuasive for the same reasons given in the Office Action dated 7/15/2003 and remarked that "this is not a rejection based on anticipation, but on obviousness." Applicants

acknowledge this statement, but as such, it does not relieve the Examiner's burden of establishing that the cited references must result in each and every element of the allegedly obvious claim (see § 2143 of MPEP, above). The Examiner attempts to finesse the requirement for employing substantially pure lysine 2,3-aminomutase in the claimed methods by blanket statements about the higher purity of proteins produced by recombinant methods, but this is insufficient to render the present claims obvious.

V. Conclusion

In view of the above amendment and remarks, reconsideration and favorable action on all claims are respectfully requested. If any issue remains to be resolved in view of this response, the Examiner is invited to contact the undersigned at the telephone number set forth below so a prompt disposition of this application can be achieved.

Respectfully submitted,

Date November 3, 2005

By _____

FOLEY & LARDNER LLP
Customer Number: 23524
Telephone: (608) 258-4303
Facsimile: (608) 258-4258

Joseph P. Meara
Attorney for Applicant
Registration No. 44,932

Written Description

Original Claims

Decision Tree

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